Comparison of *Saccharomyces cerevisiae* populations from different appellations of origin and grape varieties by microsatellite analysis

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Introduction

Fermentation technologies are deeply rooted in the history of most societies;

Winemaking environments are characterized by a fascinating genetic diversity of *S. cerevisiae* strains, but population genetic variation is still poorly understood.

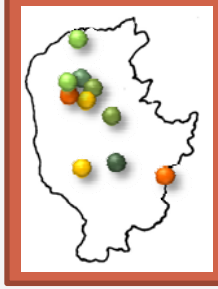
Objective

to evaluate populational relationships among *Saccharomyces cerevisiae* strains isolated from some of the Portuguese most important grapevine varieties in different appellations of origin, using polymorphic microsatellites

Materials and Methods

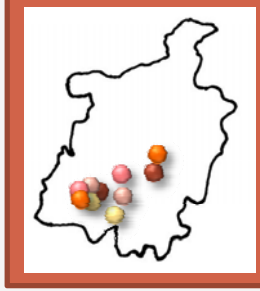
- sampling sites -

Vinho Verde



- Alvarinho
- Loureiro
- Avesso
- Arinto
- Touriga Nacional

Bairrada

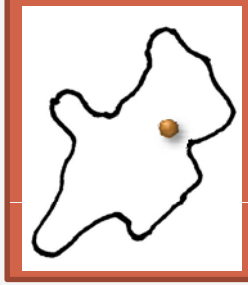


- Bical
- Baga
- Maria Gomes
- Aragonês
- Touriga Nacional

Estremadura

- Castelão
- Trincadeira Preta
- Aragonês
- Arinto
- Touriga Nacional

Palmela



- Castelão

Alentejo (sub-region Évora)

- Aragonês
- Trincadeira Preta
- Touriga Nacional

5 Wine Regions
9 Grape varieties
20 Vineyards
6 samples / vineyard

300
grape samples

Materials and Methods

- spontaneous fermentations -

Weight loss of 70 g/l:

Random collection of 30 isolates per fermentatin

Molecular typing

	Minho		*Bairrada*	*Estremadura Palmela Alentejo*	*Total*
	2001-2003	2006	2006	2006	
Nº of samples	108	84	60	48	300
Spontaneous fermentations	54	30	29	36	149
Number of isolates	1620	900	870	1080	4470
Saccharomyces sp.	100 %	100 %	100 %	9 %	

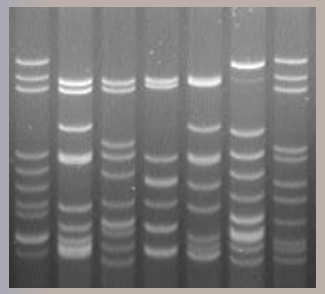
Total: 501 *S. cerevisiae* strains

Materials and Methods

- molecular typing -

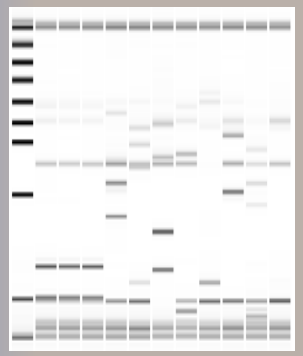
Preliminary screening

Mitochondrial DNA restriction analysis (mtDNA RFLP)



Querol et al.1992
Lopez et al. 2001

Interdelta sequence amplification



Ness et al. *1993*
Legras et al. *2003)*

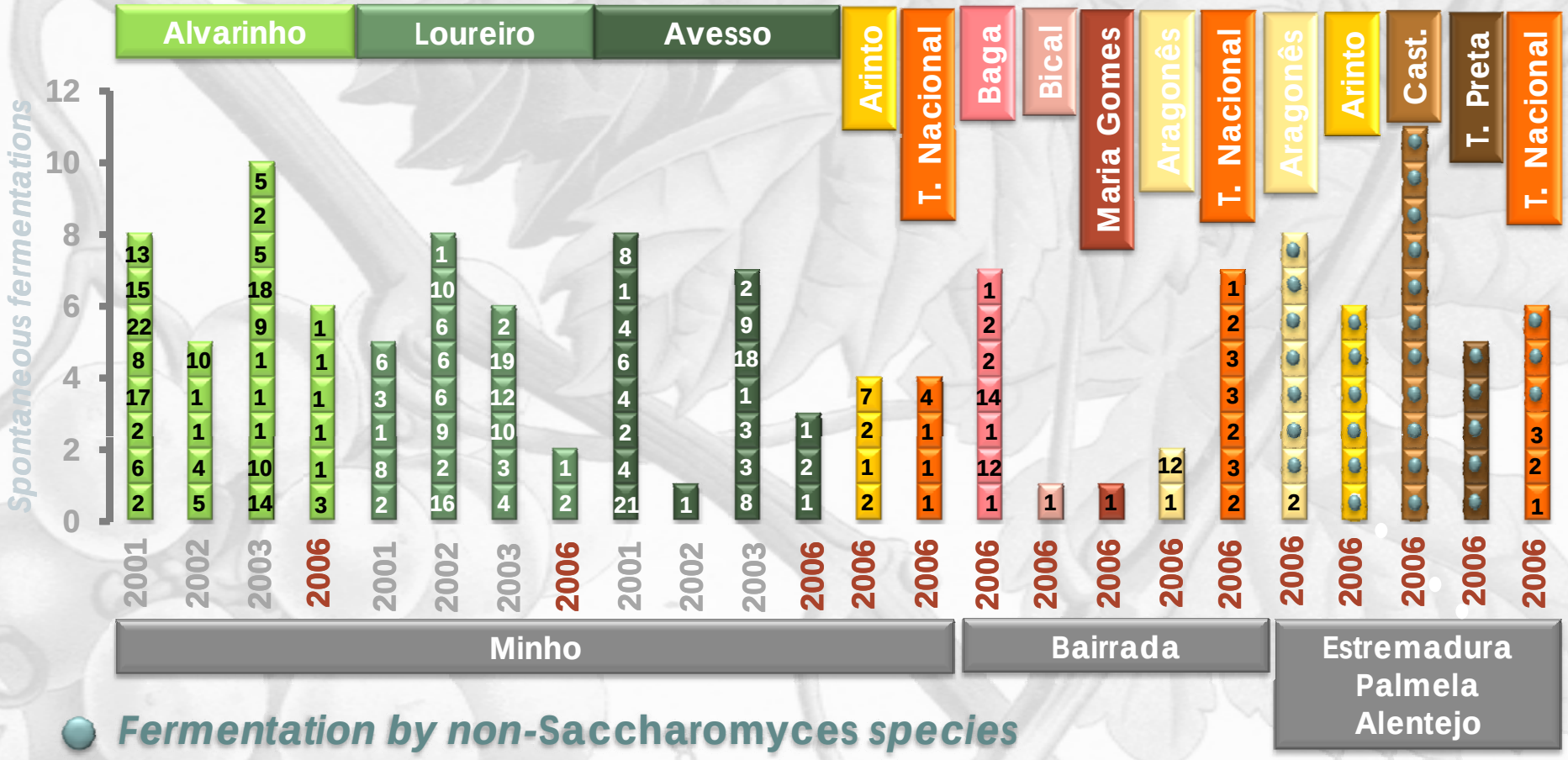
In depth characterization

Microsatelite analysis

Microsatellite	Chrom.	Position/ORF	Repeat	Ref.
ScAAT 1	XIII	86 901 – 87 129	ATT	Pérez and Gallego, 2001 Legras et al., 2005
ScAAT 2	II	YBL084c	ATT	
ScAAT 3	IV	YDR160w	ATT	
ScAAT 4	VII	431 334 – 431 637	ATT	
ScAAT 5	XVI	897 028 - 897 259	TAA	
ScAAT 6	IX	105 661 – 105 926	TAA	
YPL009	XV	YOR156c	TAA	
ScYOR267C	XV	YOR267c	TGT	
C4	XV	110 701-110 935	TAA+TAG	
C5	VI	210 250-210 414	GT	

Results

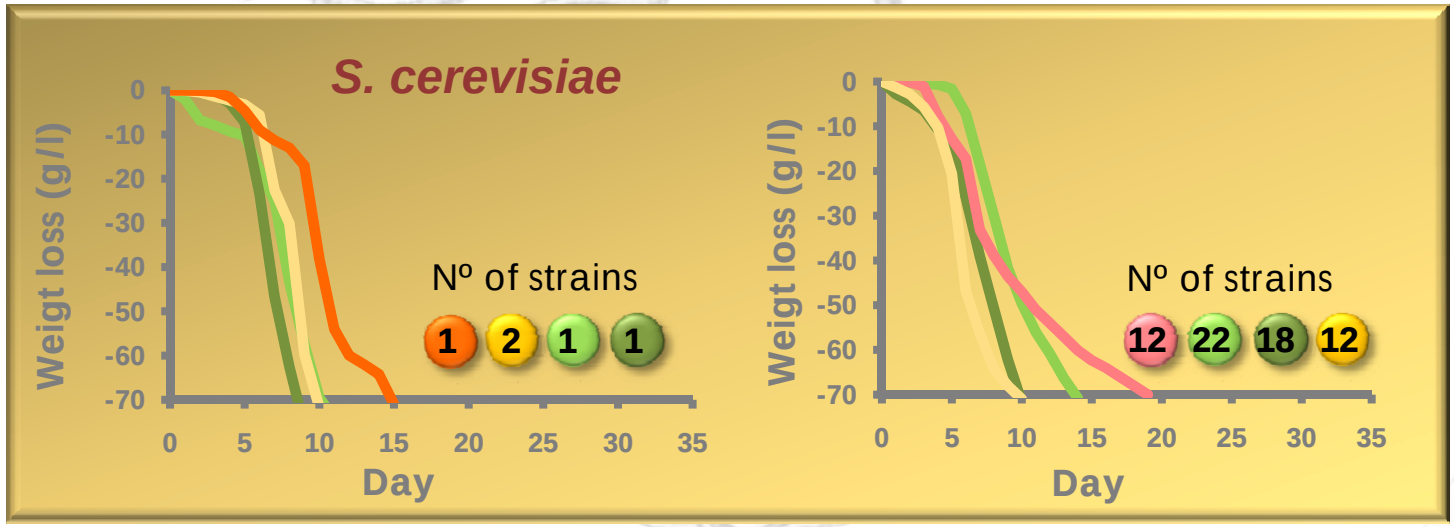
- *S. cerevisiae strains involved in spontaneous fermentations* -



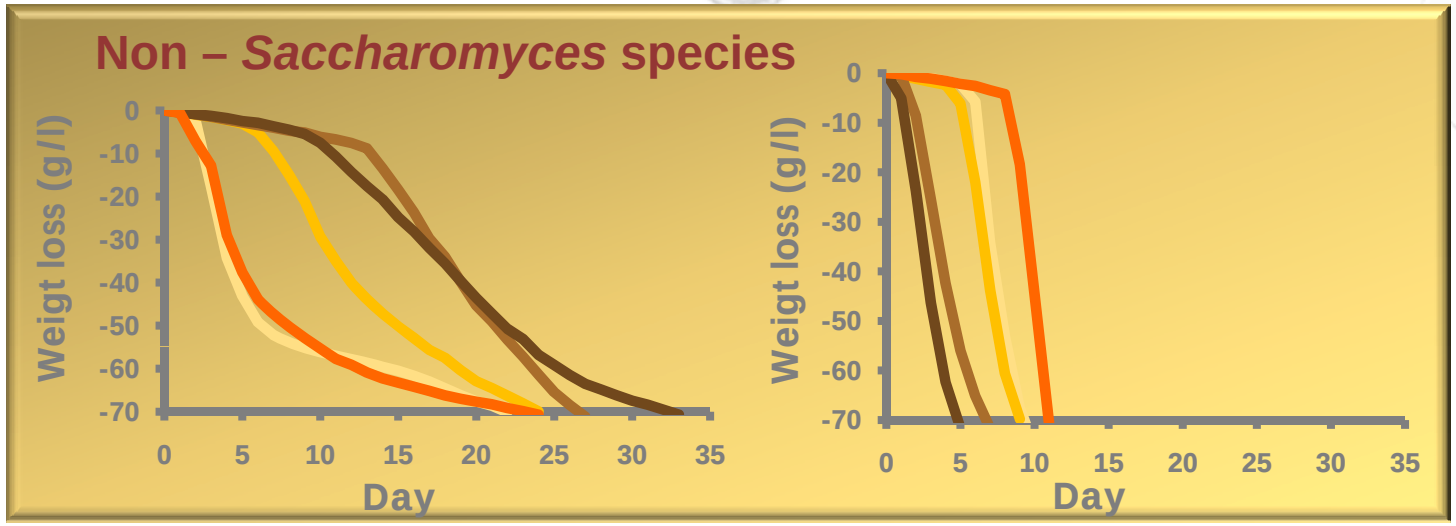
● *Fermentation by non-Saccharomyces species*

Results

- fermetation kinetics -



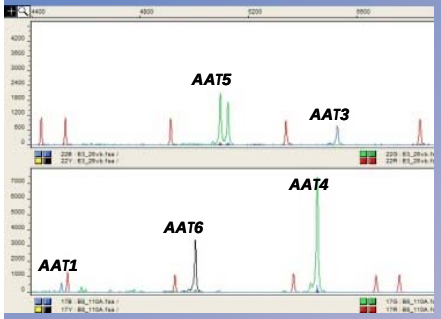
- Aragonês
- Arinto
- Touriga Nacional
- Castelão
- Trincadeira preta
- Baga
- Alvarinho
- Loureiro



Results

- distribution of the most frequent microsatellite alleles -

Most frequent alleles

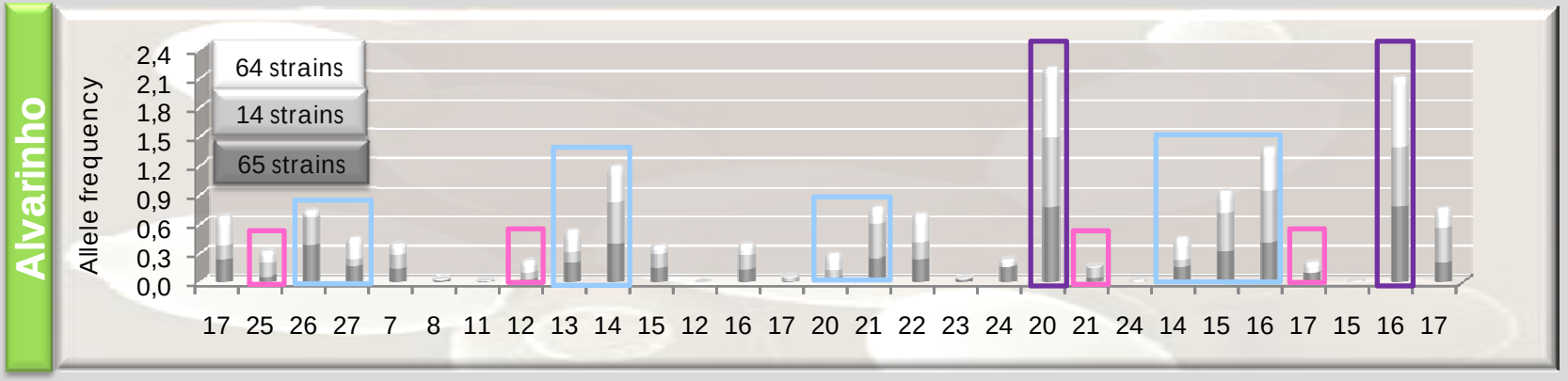


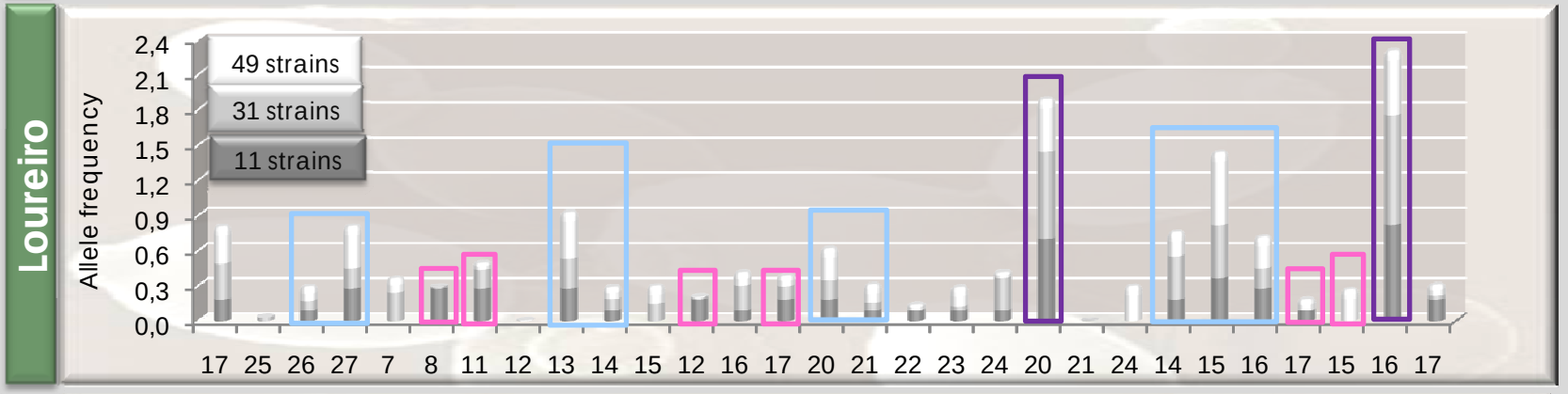
Locus	Nº of alleles	Allele (nº of repeats)
ScAAT 1	43	12 13 15 16 17 18 19 20 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 39 40 41 43 47 49 51 53 54 59
ScAAT 2	13	3 4 5 6 7 8 10 11 12 13 14 15 16
ScAAT 3	21	9 10 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 34 49 55
ScAAT 4	19	6 8 9 10 11 12 13 14 15 16 18 19 20 21 22 23 24 26 27
ScAAT 5	5	13 14 15 16 17
ScAAT 6	11	13 14 15 16 17 18 19 20 21 23 28
C4	11	20 21 22 23 24 25 26 27 36 37 41
C5	26	3 4 5 6 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 28 30 31 34
YPL009	24	43 51 53 55 57 58 60 61 62 63 65 66 69 70 71 72 73 76 77 79 80 81 82 89
ScYOR267	26	19 22 25 30 31 32 33 35 36 37 39 41 42 43 44 46 47 48 49 50 51 52 53 54 55 58

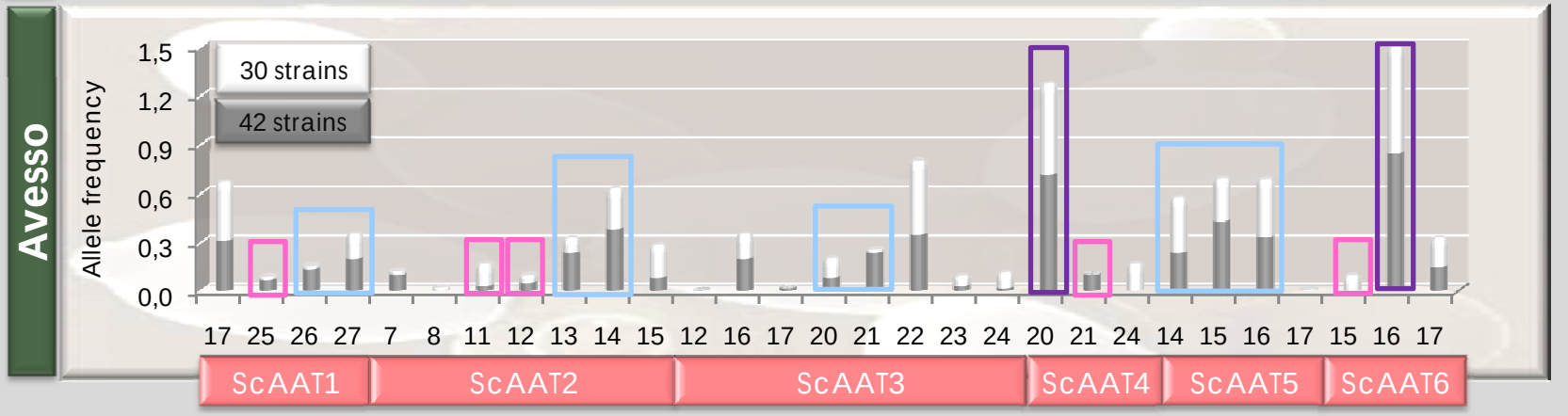
Total: 192 alleles

Distribution of the most frequent alleles among vineyards of the Vinho Verde Region in consecutive years

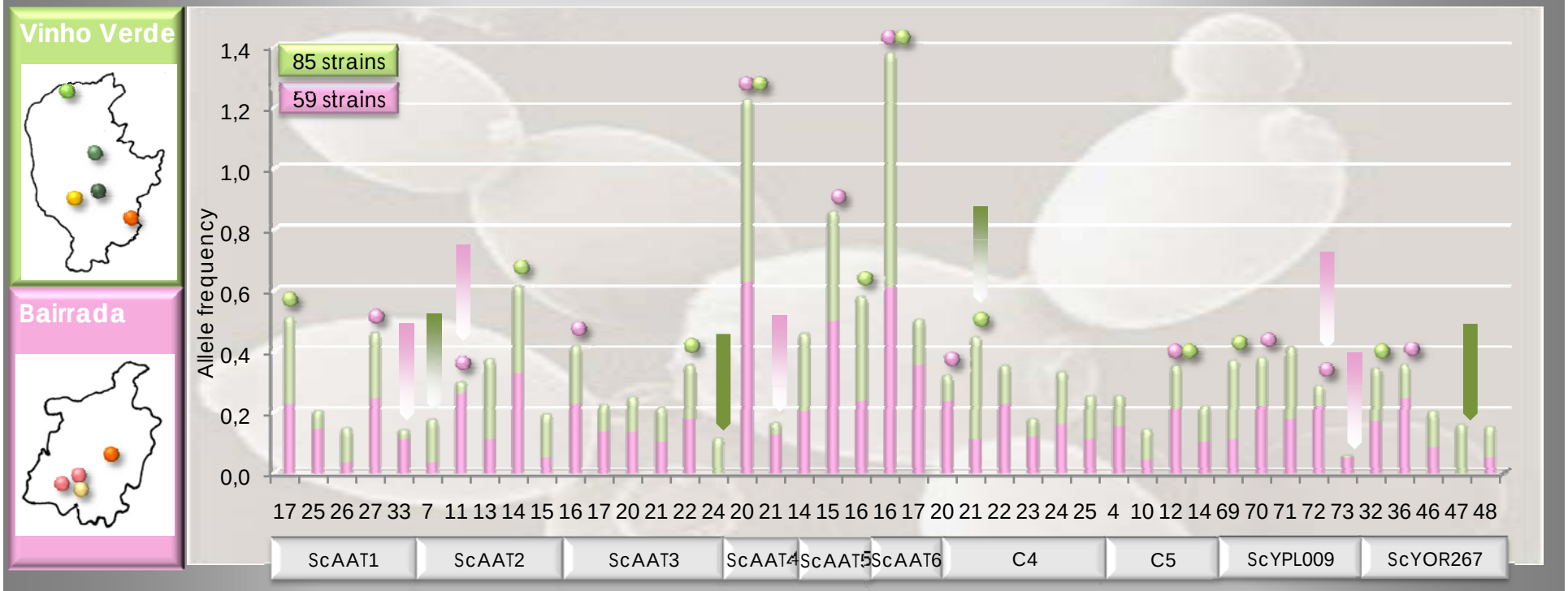
2001 2002 2003







Distribution of the most frequent alleles among S. cerevisiae strains from vineyards of the Vinho Verde and Bairrada Regions in 2006



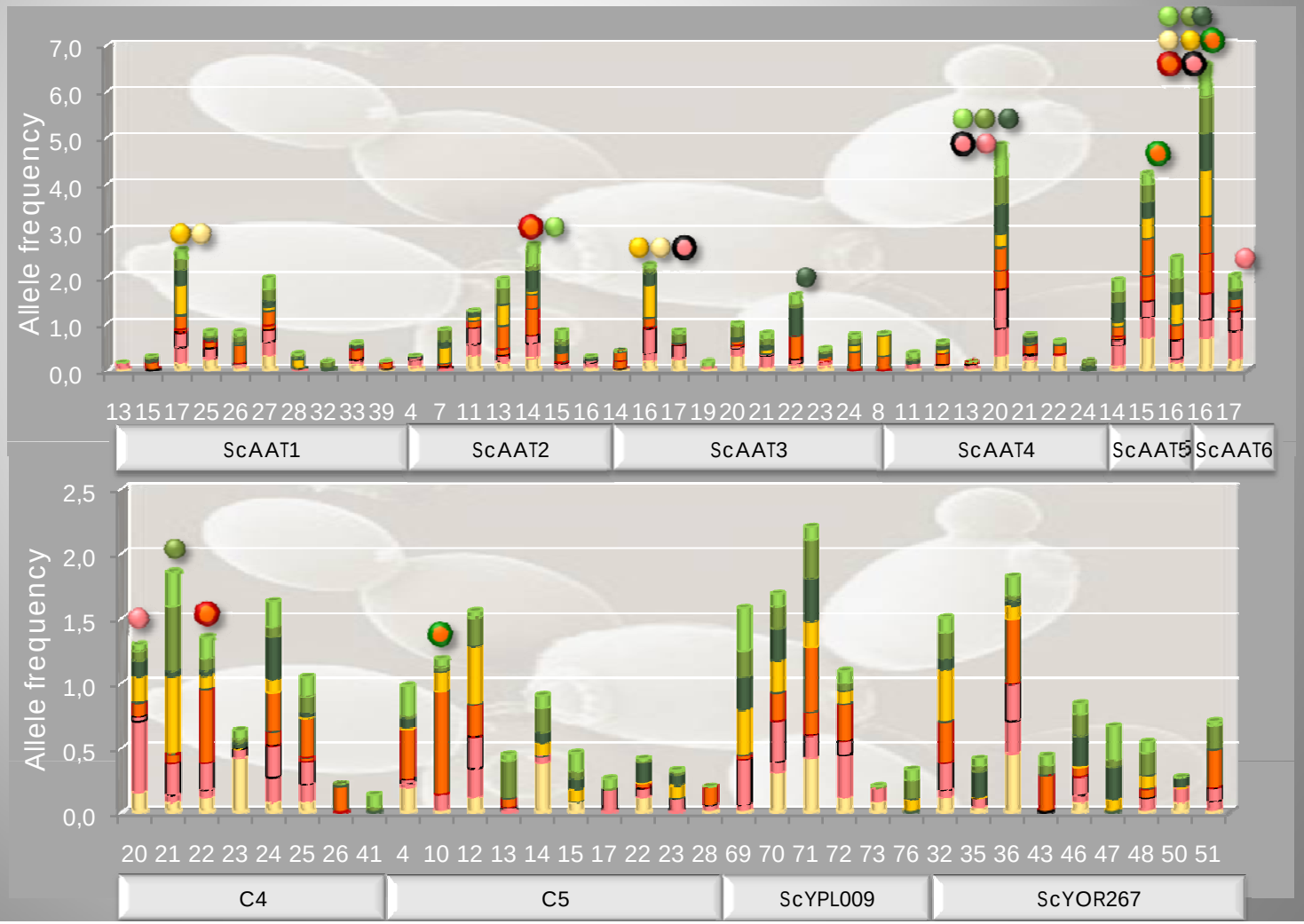
Most frequent allele per locus (Minho)

Most frequent allele per locus (Bairrada)

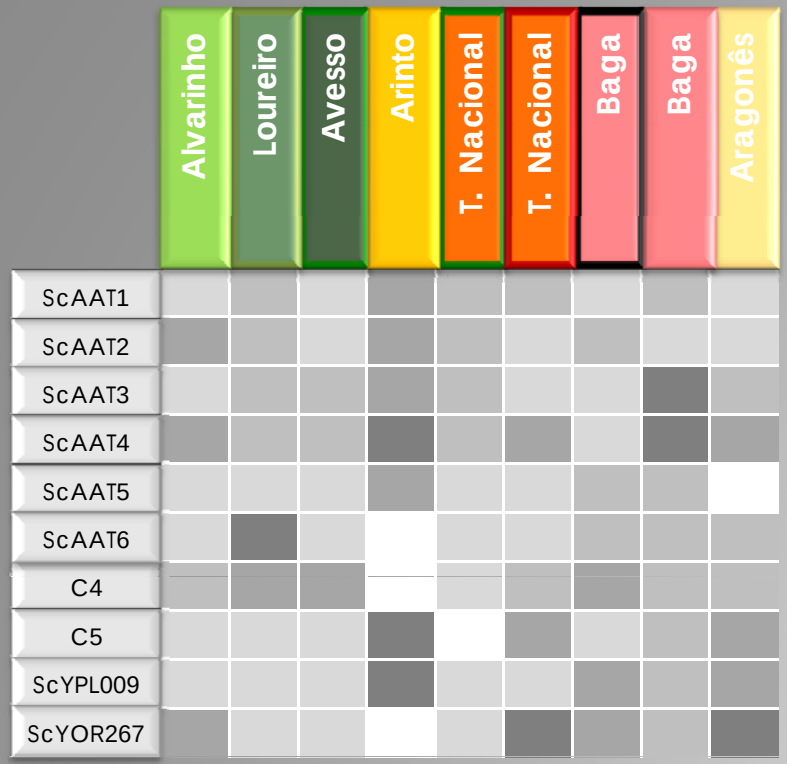
Most frequent alleles among S. cerevisiae strains from grape varieties of the Vinho Verde and Bairrada Regions

- Alvarinho (30 strains)
- Loureiro (27 strains)
- Avesso (12 strains)
- Arinto (10 strains)
- T. Nacional (5 strains) (Minho)

- Baga (18 strains) (vineyar with unique grape variety)
- Baga (14 strains) (vineyar with several grape variety)
- Aragonês (13 strains)
- T. Nacional (14 strains) (Bairrada)



Expected (He) and observed (Ho) heterozygosity



He / Ho
1 – 3
3 – 5
5 - 7
7 – 10
n.d.
(homozygous locus)

Heterozygous genotypes reduction relative to that expected under random mating is a consequence of populational substructuring

Genome renewal

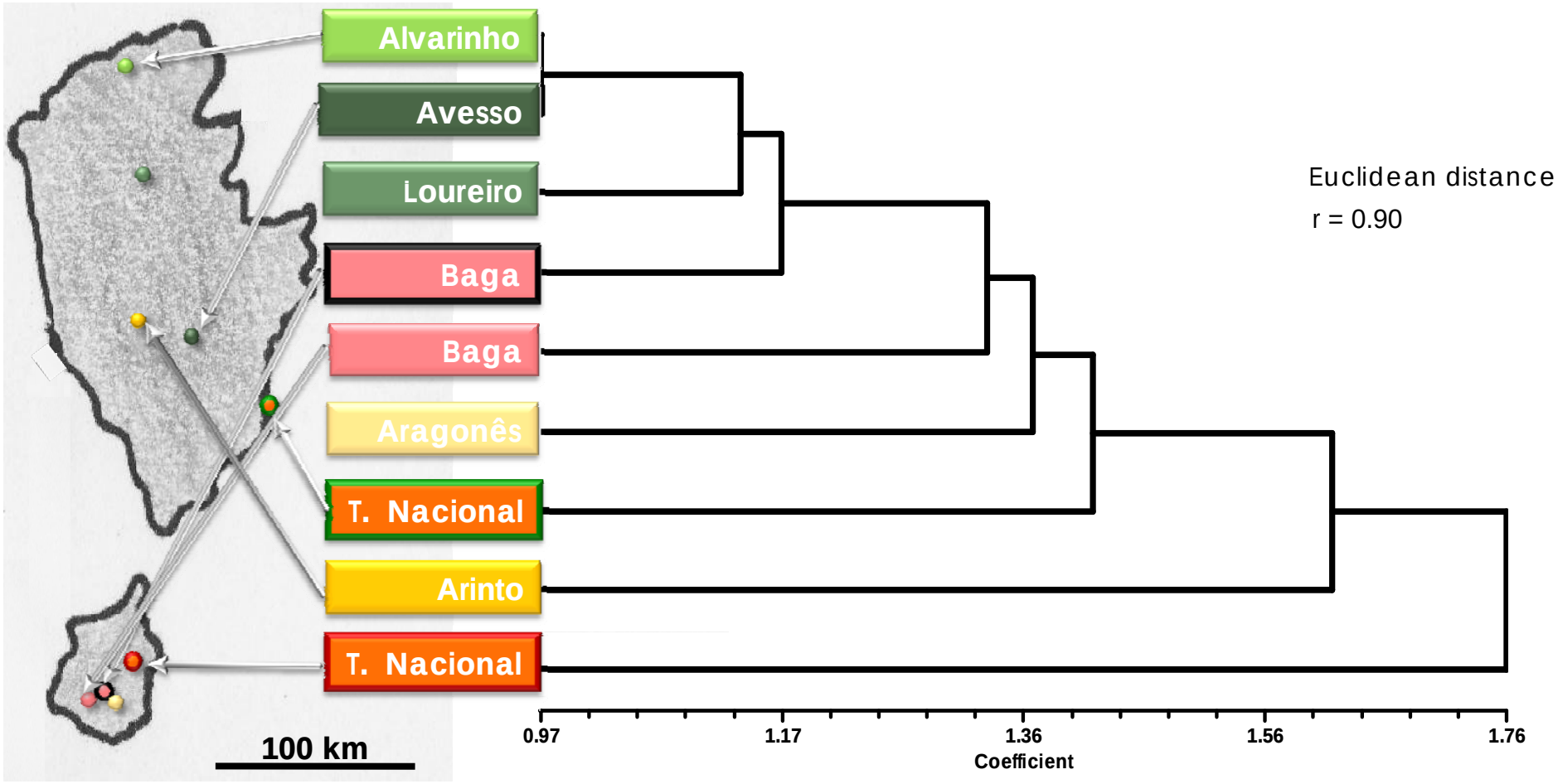
(Mortimer et al., 1994)

Environmental isolates of *S. cerevisiae* undergo mating among their progeny

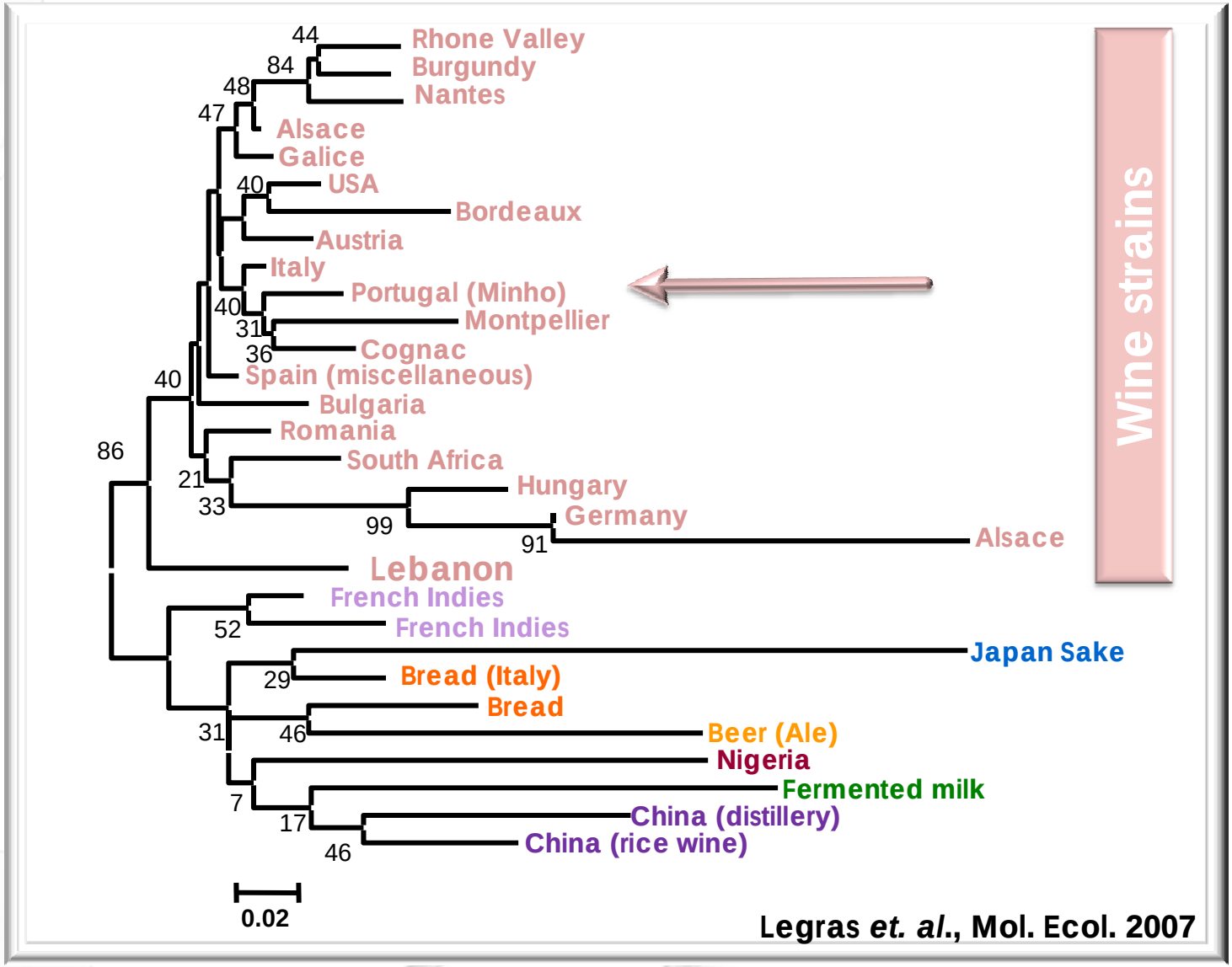
Multiple heterozygote strain

homozygote strain

Similarity of S. cerevisiae populations from distinct wine regions and grape varieties



Consensus tree of S. cerevisiae populations based on F_{ST} genetic distances



Conclusions

The finding of 501 *S. cerevisiae* strains reveals a fascinating genetic diversity in vineyard environments.

Microsatellite analysis permitted a high resolution populational screen, showing that

genetic differences among *S. cerevisiae* populations derived from both “diagnostic” vineyard-, specific alleles and the accumulation of small allele-frequency differences across ten microsatellite loci;

each vineyard contains differentiated *S. cerevisiae* populations, hypothesizing the occurrence of specific native strains that can be associated with a *terroir*.

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Quinta da Cancela
Quinta de Covela
Quinta de Lourosa
Quinta da Pedra
Quinta da Soalheira
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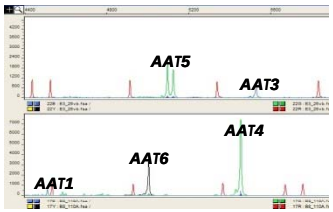
Results - Allelic distribution

Most frequent alleles



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